

## CLINICOPATHOLOGICAL STUDY OF ENDOSCOPIC GASTRIC BIOPSIES ALONG WITH THEIR H.PYLORI STATUS

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### ABSTRACT:

**Introduction :** Upper Gastrointestinal tract disorders are one of the most commonly encountered problems in clinical practice with a high degree of morbidity and mortality. *Helicobacter pylori* (*H. pylori*) is of major concern today because of its causal relationship with gastroduodenal disease. Use of flexible endoscopy has led to a marked increase in diagnostic procedures. Diagnostic endoscopy is a simple, safe and well tolerated procedure.

**Aim :** Epidemiology of endoscopic biopsies obtained from stomach and correlation with *H. pylori* status in histopathological finding of gastric lesions.

**Method :** It was a prospective study of 1 year (from 1<sup>st</sup> January to 31<sup>st</sup> December, 2016) of all patients undergoing Upper GI endoscopy for upper GI symptoms (dyspepsia, abdominal pain, vomiting, dysphagia, anemia) was conducted in Pathology department at Shri Ram Murti Smarak Institute of Medical Science College and Hospital, Bareilly.

**Result :** A total of 57 gastric biopsies were obtained. Age of the patients ranged from 15 to 81 years. Sex ratio is 2.3. Out of all biopsies, antrum was most commonly involved (70.2%) and gastritis (active, chronic and atrophic) was the most common finding (n=36/57; 63.2%) on histopathology. *H. pylori* detection rate in gastric pathologies was 16.6% with H&E, 32.7% using Giemsa which increased to reach at 49.1% when Immunohistochemistry was used.

**Conclusion :** Gastric pathologies showed gastritis (active, chronic and atrophic) as the most common finding (n=36/57; 63.2%) with antrum being most commonly involved (n=40; 70.2%). A combination of biopsy and endoscopy provides a powerful diagnostic tool for better patient management. For detecting *H. pylori*, histology is an excellent method and provides additional information about mucosa. Both Giemsa and Polyclonal *H. pylori* antibody have a very high sensitivity in detecting *H. pylori*. Since IHC is expensive, labour intensive and time consuming, it is reserved for research purposes only while Giemsa being inexpensive and less time consuming is considered sufficient for routine diagnosis of the bacteria.

**Key words :** *H. pylori*, Giemsa, Immunohistochemistry



### INTRODUCTION:

Upper Gastrointestinal tract disorders are one of the most commonly encountered problems in clinical practice with a high degree of morbidity and mortality and endoscopic biopsy is common procedure performed in the hospital for a variety of benign and malignant lesions.<sup>(1)</sup> The esophagus and stomach can be sited for a wide variety

of infections, inflammatory disorders, vascular disorders, mechanical conditions, toxic and physical reactions, including radiation injury and neoplasm.<sup>(2)</sup> Introduction of the endoscopes in 1960's has greatly improved the diagnostic facility for fiberoptic endoscopy because they are readily accessible and can easily be

sampled for specific histopathological or microbiologic investigation with available biopsy forceps. Tissue specimen can be removed from the lesions under direct vision using biopsy forceps. The procedure causes minimal discomfort and thus can be repeated. Histopathological study of biopsy specimens are used to confirm endoscopic diagnosis in suspected malignancy or to rule out malignancy in the endoscopically benign appearing lesion. The endoscopic biopsies are performed not only for the diagnosis of the disease but also for monitoring the course, determining the extent of a disease, as responses to therapy and for the early detection of complications .

Disorders of the stomach are a frequent cause of clinical disease, with inflammatory and neoplastic lesions being particularly common. Diseases related to gastric acid account for nearly one third of all health care spending on gastrointestinal (GI) diseases. Symptomatology of gastric diseases ranges from dyspepsia to altered bowel movements and dysphagia to GI bleed.<sup>(3)</sup>

*Helicobacter pylori* (*H. pylori*) is of major concern today because of its causal relationship with gastroduodenal disease. One half of the world's population has *H. pylori* infection, with an estimated prevalence of more than 90% in developing countries.<sup>(4)</sup> *Helicobacter pylori* infection is common in the Indian subcontinent. Exposure occurs in childhood and approximately 80% of adults have been infected at

some time. It is proven that *H. pylori* is the principal cause of chronic gastritis, peptic ulcer disease as well as gastric cancer.<sup>(5)</sup>

A wide range of laboratory investigations is available for diagnosis of *H. pylori*. The tests belong either to non-invasive group or invasive group. Non-invasive tests include urea breath test, serological Immunoglobulin G (IgG) and immunoglobulin M (IgM) detection, saliva and urinary antibody test, and stool antigen test.<sup>(6)</sup> The invasive tests include endoscopy-based histopathological examination by special stains or by immunohistochemistry, rapid urease test (RUT) and polymerase chain reaction. Whereas invasive tests carry high sensitivity and specificity of >90%<sup>(7)</sup>, the role of non-invasive tests, such as serology, is limited in areas of high prevalence.

Use of flexible endoscopy has led to a marked increase in diagnostic procedures involving visualisation and biopsy of the upper and lower GI tract. Diagnostic endoscopy is a simple, safe and well tolerated procedure.<sup>(8)</sup> Upper GI endoscopy, in combination with biopsy, plays an important role in the early diagnosis of GI neoplasms and provides an opportunity for a broad range of treatment options as well as potential for possible cure.

## **MATERIALS AND METHODS:**

The present study was a prospective study conducted in Pathology department at Shri Ram Murti Smarak

Institute of Medical Science College and Hospital, Bareilly, a tertiary health care centre from 1<sup>st</sup> January to 31<sup>st</sup> December, 2016 All patients undergoing Upper GI endoscopy for gastric symptoms (dyspepsia, abdominal pain, vomiting, dysphagia, anemia) were included in the study. Clearance was granted by the Ethical committee

The **Inclusion Criteria:** Patients presenting with complaints of gastric symptoms viz. dyspepsia, pain abdomen, vomiting, dysphagia, anemia and patients willing to undergo endoscopic evaluation and biopsy collection. **Exclusion Criteria:** Those with chronic liver disease with esophageal varices, patients on proton pump inhibitor therapy or any antibiotic therapy within last 1 month and patients unwilling to participate in the study.

After enrolment in the study, demographic details, clinical and medical history was obtained. Details of present illness were also obtained. All the patients underwent general and upper Gastrointestinal endoscopy examination. Routine hematological and biochemical evaluations were performed if required. The biopsy specimens were taken from the stomach and sent for histological examination. Specimen was fixed overnight in 10% buffered formalin, processed, embedded in paraffin, and cut and stained with Hematoxylin and Eosin (H&E).

H. pylori detection was done in all biopsies using H&E, Giemsa stain and IHC.

## RESULTS:

A total of 57 endoscopic gastric biopsy specimen were obtained. Age of patients ranged from 15 to 81 years. Majority of patients were in age range 31 to 60 years (71.9%). There were only 3 (5.3%) cases below 20 years and only 1 (1.8%) case aged >80 years. Mean age of patients was 47.54±16.13 years.

Majority of patients (n=40; 70.2%) were males. There were 17 (29.8%) females. Male to female ratio of study population was 2.3.

Dyspepsia was the most common clinical feature (45.6 %) followed by abdominal pain (31.6%), vomiting (26.3%), dysphagia (17.5%) and anemia (12.3%). Hematemesis and abdominal distension were relatively less frequent clinical signs and symptoms seen in 8.8% and 5.3% patients respectively.

Out of 57 stomach biopsies, antrum was most commonly involved (n=40; 70.2%) followed by body (n=14; 24.6%). There was 1 (1.8%) case each with involvement of cardiac, GE junction and pylorus regions. None of the cases had involvement of fundus.

On histopathology, Gastritis (active, chronic and atrophic) was the most common finding (n=36/57; 63.2%) followed by adenocarcinoma (n=9; 15.8%), dysplasia/carcinoma in situ (n=5;

8.8%) and signet cell carcinoma (n=3; 5.3%) respectively. There were 2 cases showing no significant changes. One case each was diagnosed as gastric polyp and MALTOMA respectively.

For *H. pylori* detection, H&E had minimum detection rate (16.6%) whereas IHC had maximum detection rate. The detection rate of IHC was significantly higher as compared to that of H&E, however, no significant difference was observed between IHC and Giemsa. For all the three techniques maximum detection was observed for active and chronic gastritis. The difference in positivity rate for different types of gastric lesions was not calculated owing to fewer number of cases.

Nature of lesions was assessed in all 57 cases. Among these 39 (68.4%) were benign and 18 (31.6%) were malignant.

## DISCUSSION:

Clinicopathological correlation of endoscopically obtained gastric biopsies has gained quite significance in view of the high mortality and morbidity potential borne by them, more so, in view of the *H. pylori* infestation showing variable nature of relationships for different types of lesions in stomach.

In present study we made an attempt to study histopathological findings of gastric lesions and *H. pylori* positivity rate in 57 endoscopic biopsy specimen obtained in our laboratory. Age of

patients enrolled in the study ranged from 15 to 81 years. Patients in age range 31 to 70 years ( 71.9 % ) were most commonly affected. Mean age of patients was  $47.54 \pm 16.13$  years. Although gastric lesions are seen in almost all the age groups, however, they are more common in middle and mature adults. Similar to observations in present study, Piatek-Guziewicz *et al.*<sup>(9)</sup> also reported majority of their patients to be above 45 years of age. Mean age of patients in different series has been reported to be above 50 years. Islam *et al.* reported mean age as 54.63 years. Similarly, Quach *et al.*<sup>(10)</sup> reported it as 54.5 years, however Memon *et al.*<sup>(11)</sup> and Trindade *et al.*<sup>(12)</sup> reported a relatively younger age profile with mean age 40 and 43 years respectively. Despite this variation in age of patients in different studies, the general age profile indicate it to be a problem more common after 40 years of age.

Majority of cases were males (70.2%). Male to female ratio was 2.3 :1. The gender profile in present study is comparable to that reported by Gulia *et al.*<sup>(13)</sup> who also reported male to female ratio as 1.74. Other studies also reported a high male dominance. Shaikh *et al.*<sup>(14)</sup> reported the male-female ratio as 1.93, Abilash *et al.*<sup>(15)</sup> reported it as 1.94. However, Memon *et al.* and Trindade *et al.*<sup>(11,12)</sup> reported a strong female predominance with male-female ratio 1:1.6 and 1:1.82 respectively.

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The present study witnessed dyspepsia (45.6 %), abdominal pain (31.6%) and vomiting (26.3%), as the three most common presenting complaints. These findings have been reported as the major clinical findings in various previous studies too.

Gastric biopsies revealed, antrum as the most commonly involved (70.2%) followed by body (24.6%). There was 1 (1.8%) case each with involvement of cardiac, GE junction and pylorus regions. None of the cases had involvement of fundus. Body and antrum of the stomach are generally prone to intestinal metaplasia in relation to chronic gastritis due to *H. pylori* infection. Contrary to findings of present study, Abilash *et al.*<sup>(15)</sup> reported was pylorus (62%) followed by fundus (29%) and body (09%) as the sites involved in gastric lesions.

Gastritis (active, chronic and atrophic) was the most common finding (n=36/57; 63.2%) followed by adenocarcinoma (n=9; 15.8%), dysplasia/carcinoma in situ (n=5; 8.8%) and signet cell carcinoma (n=3; 5.3%) respectively. There were 2 cases showing no significant changes. One case each was diagnosed as gastric polyp and MALTOMA respectively. Similar to present study, Gulia *et al.*<sup>(13)</sup> also found gastritis to be most common finding among stomach lesions (76.04%). However, Islam *et al.*<sup>(16)</sup> in their study

reported adenocarcinoma as the major finding (45.20%) and gastritis in only 20.55% cases. Contrary to this the present study had only 15.8% cases of adenocarcinoma. Jawalkar *et al.* (2015).<sup>(17)</sup> and Shaikh *et al.*<sup>(14)</sup> also reported adenocarcinoma to be higher than gastritis in their series of stomach lesions. Thus, prevalence of malignancy and gastritis might be dependent on the stage of detection and can vary from one study to another study keeping in view the fact that a prolonged precancerous process, lasting decades, precedes most gastric cancers. It includes the following sequential steps: chronic gastritis, multifocal atrophy, intestinal metaplasia, and intraepithelial neoplasia.

In present study, *H. pylori* assessment was done in 57 cases of gastric lesions biopsies. In order to attain a high sensitivity the assessment was done using Giemsa as well as IHC staining. In stomach, the cases with normal mucosa on histopathology were also negative for *H. pylori* for both the techniques. In remaining 55 cases, H&E was able to detect *H. pylori* in 9/55 (16.4%), Giemsa was able to detect *H. pylori* in 18/55 (32.7%) cases as compared to 27/55 (49.1%) cases by IHC, thus showing that IHC was a superior technique that offered a better detection of *H. pylori*. On further evaluation, it was seen that all the cases positive for *H. pylori* on Giemsa were positive on IHC too. Similar to present study, a high sensitivity of IHC as compared to traditional histopathological staining methods has also been reported in several previous

studies too. In a study by Ashton-Key *et al.*<sup>(18)</sup> reported a positivity rate of 55% for Giemsa whereas Immunohistochemistry showed a positivity rate of 66%. These findings are in close proximity with the observations of present study. Similar to findings of present study, they also found IHC to be positive in all the cases in which Giemsa was positive. Patnayak *et al.* (2009).<sup>(19)</sup> also carried out a retrospective study of one year taking endoscopic gastric biopsies from patients for histopathological diagnosis of gastritis. Microscopic sections of biopsy specimens showed features of gastritis histopathologically in routine H and E stain and where the presence of *H. pylori* was suspected were also stained with Giemsa and immunohistochemistry (IHC). Out of 29 cases, 26 (32.9%) showed presence of *H. pylori* on H and E, Giemsa and 49 (62.0%) cases demonstrated *H. pylori* on IHC stain. *H. pylori* detection by IHC had advantage over routine H and E staining..

Orhan *et al.*<sup>(20)</sup> too in their assessment found IHC to be a better technique for detection of *H. pylori* in gastric biopsy specimen when compared to other techniques such as rapid urease test and histopathology.

As far as pathologywise maximum number of positive samples are concerned, in present study, these were gastritis cases only, for which a total of 13/18 (72.2%) of Giemsa and 19/27 (70.4%) of IHC showed *H. pylori* positivity.

In study done by Gulia *et al.*<sup>(13)</sup> *H. pylori* related gastritis was reported in 75.25% cases and Trindade *et al.* (2017).<sup>(12)</sup> showed the prevalence of active *H. pylori* infection in gastric mucosal biopsies as 30.93%. These differences could be attributed to the regional difference only. Adlekha *et al.*<sup>(21)</sup> in their study showed *H. pylori* prevalence to be 62% in entire sample of upper GIT endoscopic biopsies and found it to be significantly associated with the presence of endoscopic abnormalities, peptic ulcer, and dysplasia/cancer. As compared to this the present study had a confirmed IHC for *H. pylori* in 49.1% cases. One of the reasons for this difference could be selective evaluation of specimen in different studies.

The clinical profile of cases correlated with the defined profile of patients in contemporary literature. With respect to *H. pylori* prevalence and its association with abnormalities, further studies with universal assessment of *H. pylori* are recommended.

With respect to *H. pylori* prevalence and its association, previous studies have emphasised that staining with haematoxylin and eosin is not adequate for detecting *H. pylori* and that staining with Giemsa or the use of the immunohistochemistry is the methods of choice. However, the former technique is not specific for *H. pylori* as both generate false positive results because of bacterial contaminants or debris. As it has been recognised that *H. pylori* causes gastritis and has been implicated in the

pathogenesis of gastric carcinoma and lymphoma, the importance of identifying *H. pylori* in biopsy specimens has increased.

Immunohistochemistry using an immunoperoxidase technique following heat induced antigen retrieval for detecting *H. pylori* in gastric biopsy and resection specimens is highly sensitive and easy to use. However, the modified Giemsa stain is routinely used because it is sensitive, cheap, easy to perform, and reproducible.

## CONCLUSION:

Present study was aimed to carry out a clinicopathological correlation between histopathology of gastric lesions and to assess their *H. pylori* status. For this purpose, a total of 57 endoscopic biopsy specimens were obtained and were subjected to clinicopathological assessment. Following were the key findings of the study:

Age of patients ranged from 15 to 81 years. Patients in age range 31 to 70 years (71.9 %) were most commonly affected. Mean age of patients was 47.54±16.13 years. Majority were males (70.2 %) & male to female ratio was 2.3. Dyspepsia (45.6 %), abdominal pain (31.6 %) and vomiting (26.3%) were the three most common presenting complaints.

Antrum was most commonly involved (70.2%) followed by body (24.6%). Among stomach pathologies, gastritis (active, chronic and atrophic)

was the most common finding (n=36/57; 63.2%) followed by adenocarcinoma (n=9; 15.8%), dysplasia/carcinoma in situ (n=5; 8.8%) and signet cell carcinoma (n=3; 5.3%) respectively.

Among these 39 (68.4%) were benign and 18 (31.6%) were malignant lesions.

Hence to conclude, endoscopy is incomplete without biopsy and so the combination of the two methods provides a powerful diagnostic tool for better patient management.

*H. pylori* detection rate in gastric pathologies was 16.6% with H&E, 32.7% using Giemsa which increased to reach at 49.1% when IHC was used. Despite a higher detection rate the difference between two methods was not significant statistically. Among gastric lesions *H. pylori* positivity rate was 47.1% for premalignant and malignant lesions (adenocarcinoma, Signet ring cell carcinoma and dysplasia/ carcinoma in situ) as compared to 50.0% in non-malignant/benign lesions.

For detecting *H. pylori*, histology is an excellent method and provides additional information about mucosa. Both Giemsa and Polyclonal *H. pylori* antibody both have a very high sensitivity in detecting *H. pylori*. Since IHC is expensive and labour intensive and time consuming, it is reserved for research purposes only while Giemsa being inexpensive and less time consuming is considered sufficient for routine diagnosis of the bacteria.

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**TABLES, FIGURES & GRAPH:**

**Table 1: Age Profile (n=57)**

Age Group	No.	%
11-20 Yrs	3	5.3
21-30 Yrs	9	15.8
31-40 Yrs	8	14.0
41-50 Yrs	12	21.1
51-60 Yrs	12	21.1
61-70 Yrs	9	15.8
71-80 Yrs	3	5.3
>80 Yrs	1	1.8
Total	57	100
Mean Age±SD (Range in years)		47.54±16.13 (15-81)

**Table 2: Clinical Profile (n=57)**

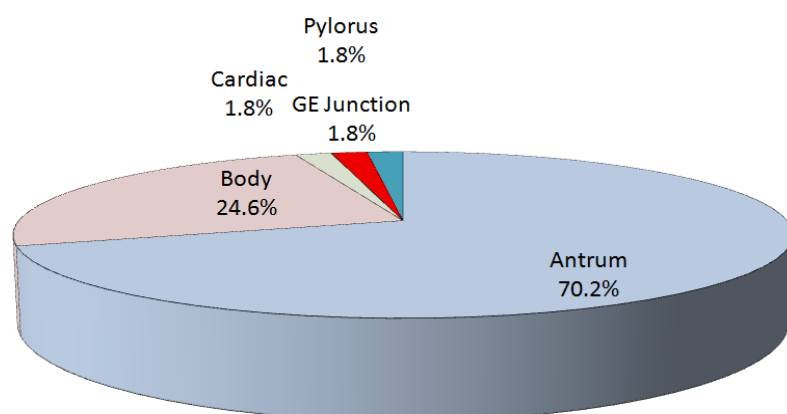
Sign/Symptom	No.	%
Dyspepsia	26	45.6
Abdominal pain	18	31.6
Vomiting	15	26.3
Dysphagia	10	17.5
Anemia	7	12.3
Hematemesis	5	8.8

Abdominal distension	3	5.3
Diarrhoea	1	1.8

**Table 3: Gastric Pathologies (n=57)**

Pathology	Antrum (n=40)	Body (n=14)	Cardiac (n=1)	GE Junction (n=1)	Pylorus (n=1)	Total
No significant change	1	1	0	0	0	2
Active gastritis	5	2	1	0	0	8
Chronic gastritis	22	5	0	0	0	27
Atrophic gastritis	1	0	0	0	0	1
Gastric polyp	1	0	0	0	0	1
Dysplasia/Ca in situ	3	2	0	0	0	5
Adenocarcinoma	6	1	0	1	1	9
Signet cell carcinoma	1	2	0	0	0	3
Maltoma	0	1	0	0	0	1

$\chi^2=26.14$  (df=32); p=0.757



**Table 4: Detection of *H. pylori* – summary of the results obtained by Giemsa and IHC methods (n=55)**

SN	Specimen type	H&E	Giemsa	IHC
1.	Active gastritis(8)	3(37.5%)	4 (50%)	5 (62.5%)
2.	Chronic gastritis(27)	5(18.5%)	9 (33.3%)	14 (51.9%)
4.	Gastric Polyp(1)	0	0	0
5.	Dysplasia/Ca in situ(5)	0	2 (40%)	2 (40%)
6.	Adenocarcinoma(9)	1(11.1%)	2 (22.2%)	4 (44.4%)
7.	Signet ring cell carcinoma(3)	0	1 (33.3%)	2 (66.7%)
8.	Maltoma(1)	0	0	0
	Total	9(16.6%)	18 (32.7%)	27 (49.1%)

H&E vs IHC (Total):  $\chi^2=13.4$  (df=1); p<0.001

Giemsa vs IHC (Total):  $\chi^2=3.05$  (df=1); p=0.081

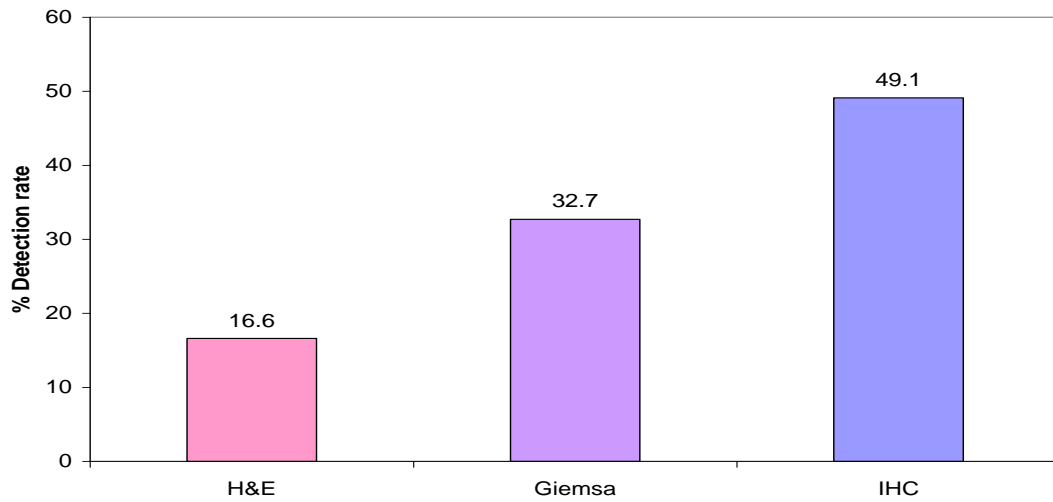


Fig. 3: Detection of *H. pylori*-Comparison between H&E, Giemsa and IHC

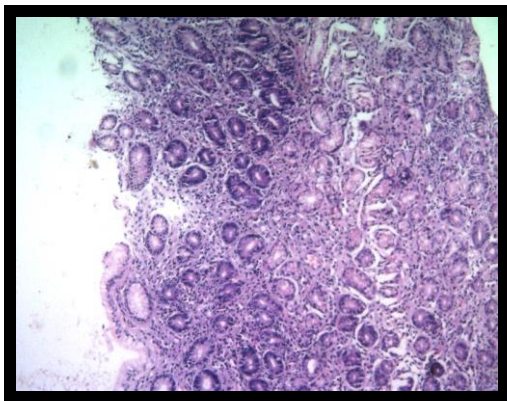


Photo Plate 01 : Active Gastritis with *H. pylori* (H& E, 10x)

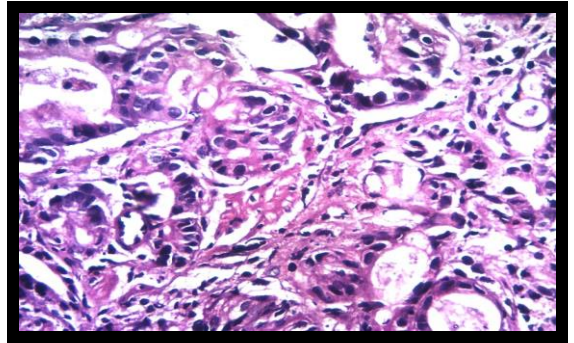


Photo Plate 02 : Adenocarcinoma, Intestinal type (H&E, 40x)

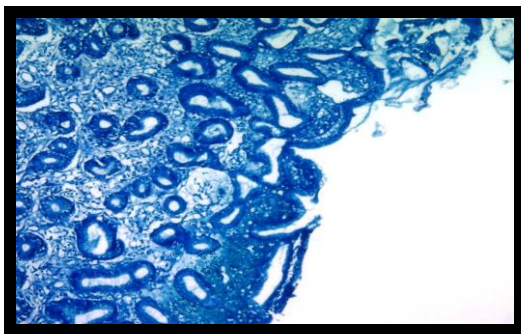


Photo Plate 03 : Chronic Gastritis with *H. pylori* (Giemsa, 10x)

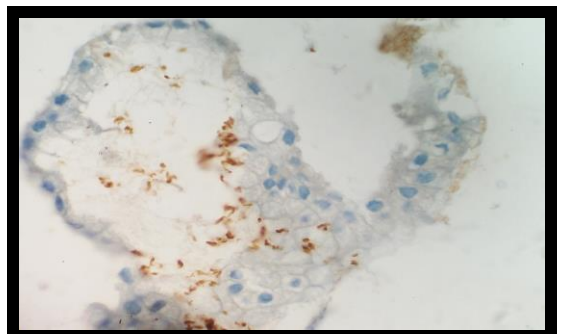


Photo Plate 04 : Chronic Gastritis with *H. pylori* ( IHC for *H. pylori*, 40x)